MOVEMENT PATTERNS OF THE NATIVE, *PROCAMBARUS CLARKII*, (CRUSTACEA: DECAPODA) IN WEST-CENTRAL TEXAS

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ABSTRACT

This study was undertaken to determine movement patterns and burrow occupancy of the red swamp crayfish, *Procambarus clarkii,* at Anson Springs in Tom Green County, Texas. This study represents the first use of radio telemetry within *P. clarkii*'s native range. Eight specimens (three males and five females) were fitted with radio transmitters and their locations were tracked in-stream six times a day, from August 10–24, 2017. Results suggest that reproductive males undertake fewer movements than females during the day and the night. Also the majority of movements for both males and females occur after 1800. Burrow occupancy suggests that males are more often found in bank burrows while both males and females are less often observed under boulders.

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INTRODUCTION

The crayfish of North America are divided into two families. The family Astacidae is found only in the drainages of the northern Pacific coast of the United States and the southern Pacific coast of Canada. The second family, Cambaridae, contains all crayfish in North America east of the Rocky Mountains including those found in Central America and the Caribbean (Crandall & De Grave, 2017). The family Cambaridae is characterized by the ability of the species to switch between a reproductive and non-reproductive state between successive molts after attaining adulthood. The family Astacidae does not possess this life history character (Hobbs Jr., 1942).

Members of the family Cambaridae are able to change their reproductive state by molting between a breeding form (Form I) and a non-breeding form (Form II). Form I males can be identified based on an increased pliability of the projections on their gonopods used to manipulate a spermatophore into the female's sperm storage area known as the annulus ventralis. Form II males have hardened, cornified gonopods incapable of spermatophore manipulation thus cannot successfully reproduce. Form I males also have enlarged hooks, greatly diminished on Form II males, on their third ishiopodite (walking leg) which is used to subdue females during copulation. Females are also considered to have a breeding and non-breeding form which is determined by the presence of enlarged glair glands which secret the mucus-like fluid used to facilitate egg fertilization. Additionally reproductive females have elongate pleopods on the abdomen, or the presence of eggs/juveniles still attached to the female's abdomen (Payne & Price, 1983; DiStefano et al., 2012)

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Crustaceana

In addition to the family Cambaridae having a unique reproductive biology. Crayfish in general are unique among freshwater decapods in that they are both ecologically and economically important. They are ecologically important predators, bioprocessors, and serve as prey items for both aquatic and terrestrial organisms (Hobbs III, 1993; Taylor et al., 2007). Economically, crayfish support a worldwide multi-million-dollar human food industry (Huner, 2002).

Despite the importance of crayfish for both humans and ecosystems alike, it is estimated that 32 percent of the world's almost 600 described species of crayfish are threatened with extinction (Richman et al., 2015). In the United States and Canada alone, threatened species have been estimated to be around 48 percent. The threats to biodiversity are exacerbated by a lack of basic ecological information on 60 percent of the crayfish species in the United States and Canada which precludes effective management of imperiled species (Taylor et al., 2007). Moore et al., (2013) states that the biology and life history have been formally described for only 12 percent of United States and Canadian crayfish. This lack of fundamental basic and applied ecology represents a major problem in assessing the status of individual species.

Within the United States, the state of Texas has been largely overlooked by crayfish researchers. The Pecos, Lower Rio Grande, Southern Plains, and West Texas Gulf ecoregions have formal life history descriptions for only half of the native crayfish fauna, less than most other parts of the United States and Canada that have comparable crayfish diversity. There are two primary reasons for this: 1) much of current and previous crayfish research is centered around systematics, so areas of relatively low diversity, such as the aforementioned drainages, are overlooked by astacologists, and 2) Texas has so far failed to recognize the importance of basic crayfish research and consequently failed to support and fund research initiatives in this area like other states (DiStefano, unpubl.). As a consequence, the amount of crayfish diversity in Texas remains unclear.

The most recent peer-reviewed survey of the state of Texas was published more than half a century ago and reported only 18 species of crayfish within the boundaries of the state (Penn & Hobbs, 1958). A more recent estimate by the Carnegie Museum of Natural History reports 43 species within the state (Fetzner & Johnson, 2011). Eight new species have been described from Texas since 2008 (Johnson, 2008, 2010, 2011a, 2011b). The 1952 study also did not extensively survey much of the western half of the state including the Concho River system. According to the museum records however, the Concho River is home to two native crayfishes with one of which being the red swamp crayfish, *Procambarus clarkii* (Girard, 1852).

The red swamp crayfish was originally described as *Cambarus clarkii* (Girard 1852). The type specimen was collected by John H. Clark during his time stationed as a naturalist with the United States-Mexican Boundary Commission. The specimen was sent to Charles Girard and was published in the Proceedings of the Academy of Natural Sciences of Philadelphia in 1852. The type-locality for this specimen was given as "Between San Antonio (Texas) and El Paso del Norte" (Girard 1852). This original morphological description was lacking so the holotype was re-described by Hagen in 1870.

Procambarus clarkii is native to the south-central United States and north-eastern Mexico. In the United States it is known to be native in 12 states: Alabama, Arkansas, Florida, Illinois, Indiana, Kentucky, Louisiana, Mississippi, Missouri, Oklahoma, Tennessee, and Texas (Taylor et al. 2007). In northern Mexico *P. clarkii*'s original native range was most likely in tributaries of the Rio Grande in the states of Chihuahua, Coahuila, and Nuevo Leon (Alvarez & Villalobos 2015). It has since expanded its range, due to habitat alteration and aquaculture related introductions, into 22 US states (Nagy et al. 2018) and five Mexican states (Campos & Rodríguez-Almaraz, 1992; Hernández et al., 2008; Torres & Alvarez, 2012) outside its native range (Figure 1). *Procambarus clarkii* has also been introduced into an ever-growing list of countries (Figure 2) in Africa, Asia, Europe, and Central and South America (Loureiro et al. 2015). These introductions are due to *P. clarkii*'s importance as an aquaculture species which has led to its status as an invasive pest species in areas where introduced (Hobbs et al., 1989).

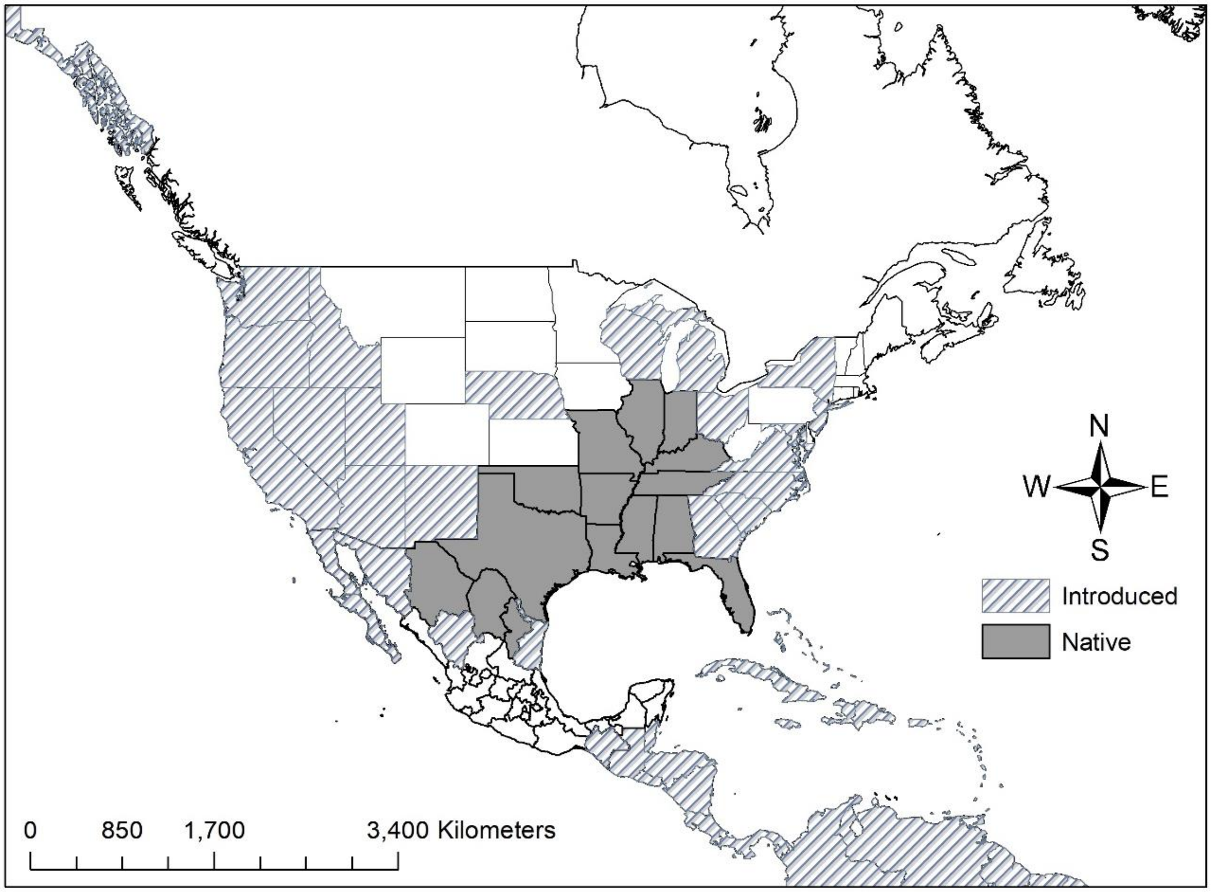

Figure 1: Map displaying the native range (solid) and the introduced range (bars) of the red swamp crayfish, *Procambarus clarkii*, in North America, South America, and the Caribbean.

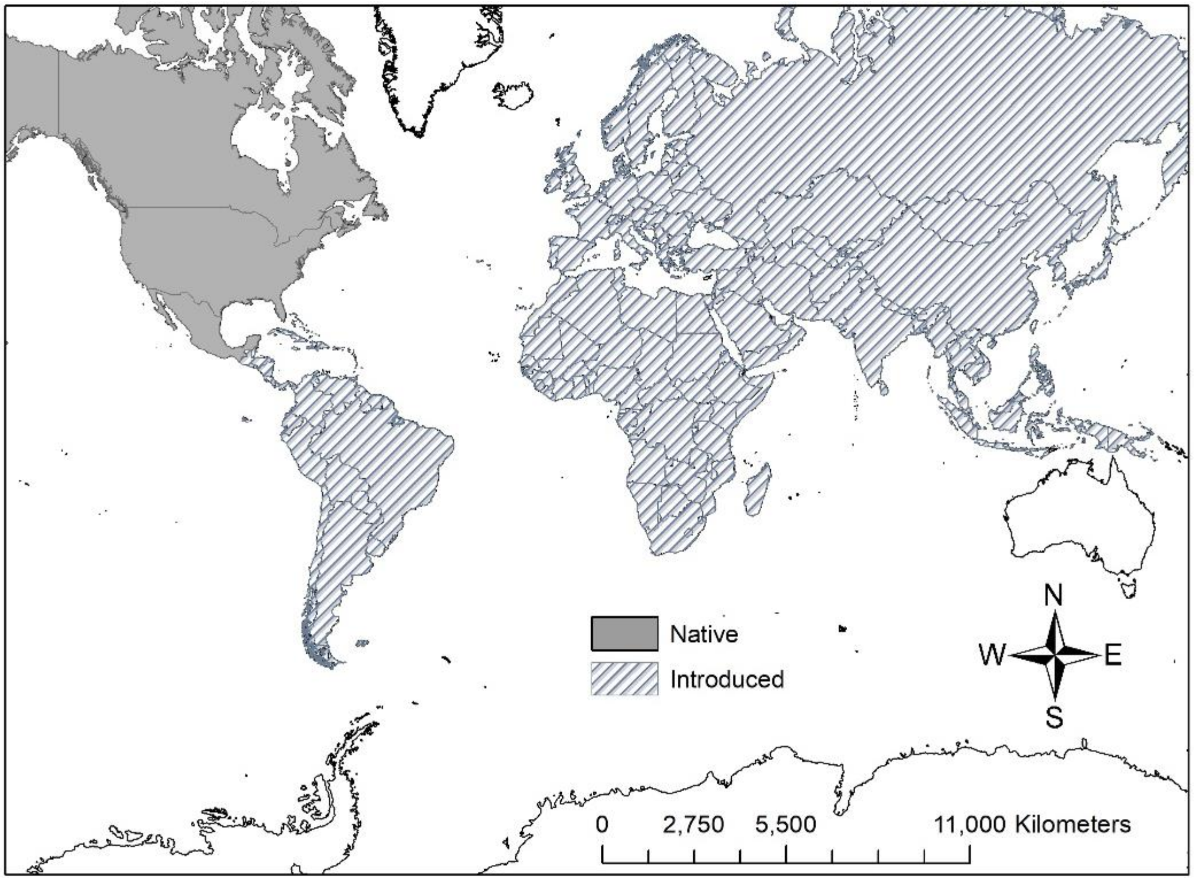

Figure 2: Map depicting where the red swamp crayfish, *Procambarus clarkii*, is native (solid) and introduced (bars).

The importance of *P. clarkii* as an invader and human food source has resulted in a well-studied life history (Huner & Barr, 1991) however basic ecology and behavioral studies have focused on invasive populations in Europe rather than native populations in North America (Barbaresi et al. 2004). The South Concho River provides an area suitable for the study of *P. clarkii* within its native range and near the type locality given for this species by Girard (1852). The data gathered from an ecological study in the native range of *P. clarkii* would appear to provide insight on management practices of this species across the globe.

Objectives:

1. Determine daily movement patterns of the red swamp crayfish, (*P. clarkii)* within its native range and habitat.
2. Determine any difference in use of refugia between the sexes of *P. clarkii*.

MATERIALS AND METHODS

Study Site

The study site selected is a 61-meter reach of the South Concho River known as Anson Springs. Anson Springs is the head spring of the South Concho River, a tributary of the Concho River, and is located approximately 6.6 kilometers south of Christoval, Texas, on the Head-of-the-River Ranch in Tom Green County, Texas. The spring has perennial flow and is fed from a perched, limestone aquifer in the Upper Edwards Plateau ecoregion of Texas (Brune, 1975). The immediate area around the spring is a secondary, deciduous forest with an overstory of *Carya spp.*, *Juglans spp.*, *Quercus spp.*, and *Ulmus spp.* The understory is dominated by *Smilax spp., Boehmeria spp.,* and various grasses. The spring resurges from a series of fissures in the limestone bedrock which makes up the majority (>50%) of the oligotrophic, aquatic area. The remainder of the aquatic area is characterized by boulders (inorganic features >250 mm), detritus (largely course particulate organic matter, CPOM, from the riparian zone), and emergent vegetation with the latter being relatively sparse. A dam of anthropogenic origin served as the downstream terminus for the study area.

Data Collection

Aerial photographs were taken with a GoPro Hero 4 Black camera (San Mateo, California) that was attached to a painter's pole to acquire high resolution photography of the river 2.44 meters above water level. A scale map was produced using the photographs of the stream in Adobe Photoshop CC 2018 (San Jose, California). First the image distortion was corrected for using the fisheye correction feature. Next the images were manually aligned and merged using the blend tool to create a seamless, scaled map of the study area.

Eight crayfish were collected using dipnets, then the point of capture was marked instream with flagging tape tied to small stone and on the scaled map. The crayfish were placed in a holding container until the carapace was dry (approximately 30 minutes). Upon drying the total carapace length (TCL) and weight after drying were measured. Sex and reproductive condition were determined based on gonopod morphology for males and pleopod length for females. In an attempt to keep size of the animals constant, only the largest individuals were used in the study (Table 1) with similar carapace lengths (mean ± SD; Male TCL: 43.4 ± 3.5 mm; n= 3, Female TCL: 41.8 ± 2.2 mm; n=5). Crayfish were fitted with BD-2 radio transmitters (0.75 g, Holohil Systems Ltd., Ontario, Canada) on the dorso-lateral surface of the carapace using cyanoacrylate glue. The adhesive was then allowed to dry and the crayfish were released at the point of capture. Marked crayfish were tracked to their location in stream using a S1000 radio receiver (Communication Specialist, Inc., Orange, California) equipped with a three-element folding yagi antenna (Advanced Telemetry Systems, Isanti, Minnesota). Crayfish were tracked using homing to their specific location instream at 0000, 0600, 0900, 1200, 1600, and 1800 hours beginning on August 10th and continuing through August 24th. All night time tracking was done with the assistance of artificial light sources. Radio transmitters will not become permanent attachments on the crayfish, but rather will be shed during natural replacement of the exoskeleton.

Crayfish	Transmitter Frequency (mHz)	Sex	TCL (mm)	Weight (g)
a	151.410	Male	41.2	21.53
b	151.490	Female	44.0	21.39
c	151.510	Male	47.4	27.53
d	151.570	Female	43.8	22.21
e	151.590	Male	41.5	21.00
f	151.649	Female	41.3	17.85
g	151.810	Female	41.1	15.80
h	151.590	Female	38.7	15.02

Table 1: Table denoting crayfish (a-h), transmitter frequency in megahertz, sex, total carapace length (TCL) in millimeters, and weight in grams.

Data Analysis

Recapture points were transferred from the map to the digital image in Adobe Photoshop CC 2018. A section of the dam in river was measured to be 28 cm wide which converted to 126 pixels on the digital image. Distances between recaptures were calculated using the custom measure tool.

Movement Patterns

Mixed Effects Regression models were created using the lme4 (ver. 1.1-17) of the open-source program, R (R Development Core Team 2018) for rate of movement (cm/hr) among individuals of differing sex and at differing time classes. Sex was broken into "Male" or "Female". Time classes were broken into "Day" (0900; 1200; and 1800) and "Night" (2100; 0000; and 0600). Each model was examined for the best fit using Akaike Information Criterion (AIC). The best fit model was selected as the one with the lowest AIC value if ΔAIC >2.

Refugia Preference

Data on refugia type was collected for each location attempt and placed into three categories of burrow type as well as in the open if individuals were not observed using refugia. The categories included bank, a burrow dug into the alluvium along the edge of the stream; boulder, a burrow found under an inorganic stream feature >250 mm; and organic, which included emergent vegetation and detrital material such as course woody debris, leaf litter or other CPOM. Burrow type was compared between sexes using a generalized linear mixed effects regression model fitted by maximum likelihood, within the lme4 package (ver. 1.1-17) of the open-source program R, to account for repeated sampling. To do this the crayfish ID was the random effect and the sex of each individual was the fixed effect. Each burrow type was a true/ false statement so either a male or female was found in a bank, or not; a boulder, or not; or organic material, or not. Environmental data was not collected due to the static nature of a spring environment.

RESULTS

Movement Patterns

Between August 10th and August 24th, a total of 200 consecutive recapture points for both male and females were logged in the study which were used to calculate average rate of movement (Mean ± Standard Deviation; Male: 17.31 ± 54.43 cm/hr and Female: 49.72 ± 100.41 cm/hr). The average rate of movement for both sexes was between 2- 36.34 meters/day (Table 2). When rate of movement between “Night” and “Day” classes were compare for both males and females it was observed that both males and female moved faster at night than during the day (Male: ΔAIC= 9.86; Figure 3; Female: ΔAIC= 16.57; Figure 3).

When rate of movement was compared between males and females at night, it was observed that females moved faster at night than did males (ΔAIC=8.28; Figure 3). It was also observed that females moved faster in the day than did males (ΔAIC=6.75, Figure 3).

Average Rate of Movement (Meters/Day)	Standard Deviation (±)	Crayfish ID	Sex
1.99	7.91	a	M
8.99	13.83	b	F
2.03	3.93	c	M
7.16	13.42	d	F
24.95	32.85	e	M
3.94	10.69	f	F
14.62	19.75	g	F
36.34	51.83	h	F

Table 2: Table depicting Average Rate of Movement (meters/day) for each crayfish in the study.

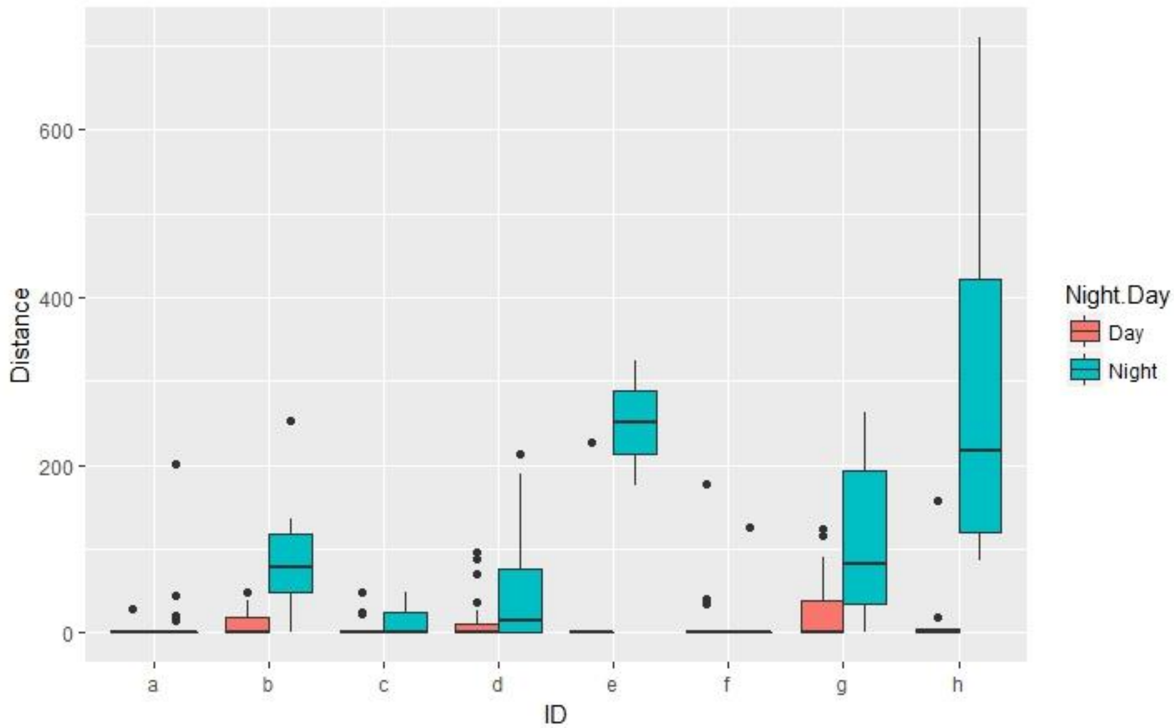

Figure 3: Box plot of the daytime (Red) and nighttime (blue) for each crayfish in the study.

Refugia Preference

Throughout the study crayfish were observed in burrows 225 times and in the channel only 45 times. Crayfish were observed in bank burrows 164 times, boulder burrows 38 times, under organic material 23 times, and in the open 45 times (Figure 4). When male and female burrow preference was analyzed for all three burrow types it was observed that males were more likely to be observed in bank burrows, while females showed no indication that banks were preferred burrows (Male: $SE=2.0$; $z=2.2$; $p<0.05$, Female: $SE=1.3$; $z=-1.3$; $p=0.21$). It was also found that both males and females were less likely to be observed in boulder burrows than other burrows (Female: $SE=0.60$; $z=-2.8$; $p<0.005$, Male: $SE=1.22$; $z=-2.0$; $p<0.05$). Finally, the sample size was too small to determine preference for individuals found in the open. Males were not observed using organic material during the duration of this study.

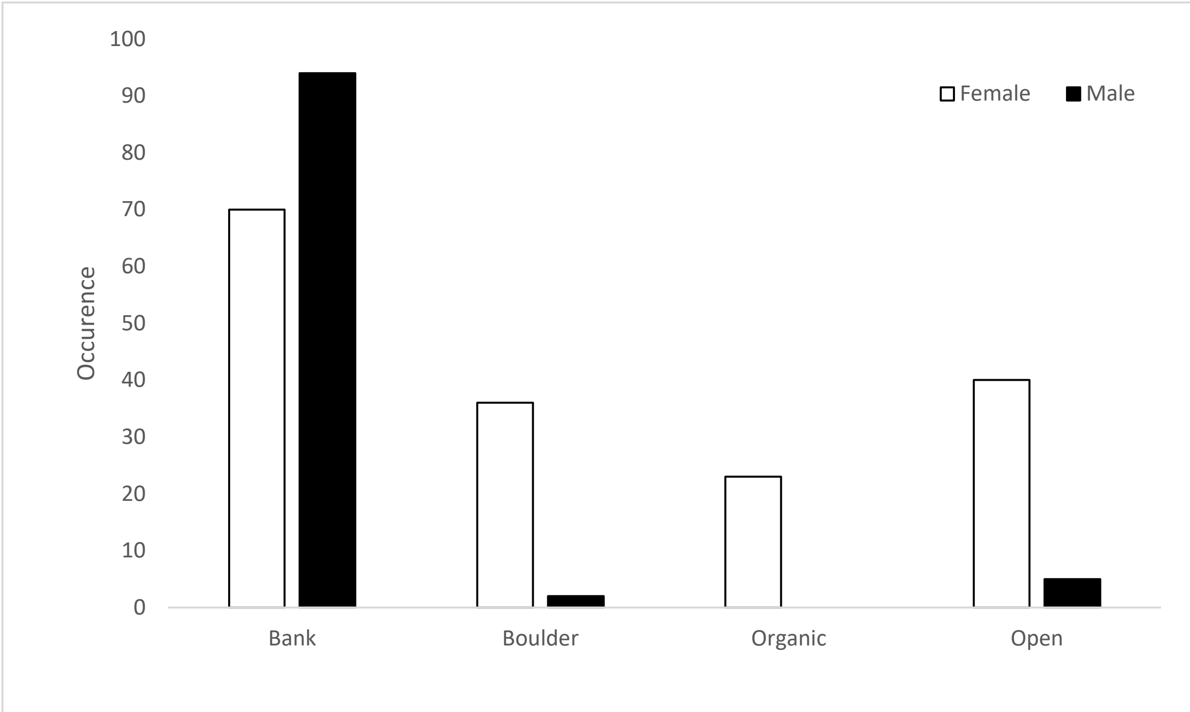

Figure 4: Graph depicts the occurrence of males in blank and females in white in the four habitat types in which they were observed.

DISCUSSION

Individuals of the population of *P. clarkii* at this study site appear to represent the main detritovores for that section of the river. This can be assumed because they are also the most robust invertebrate within the spring. While being considered a pest for the areas where introduced, this characteristic makes *P. clarkii* an important member of the food web within its native habitat.

Previous studies have been conducted using radio telemetry on the movement patterns of introduced populations of *P. clarkii* in southern Europe. Gherardi et al. (2002) tracked seven males and seven females in a temporary stream in Portugal. Barbaresi et al. (2004) tracked five females and five males in irrigation ditches around Florence, Italy. Aquiloni et al. (2005) tracked another five female and five males in a different temporary stream in Portugal. The rate of movement for both sexes observed by Gherardi et al. (2002) was between 1-11 m/d, and Aquiloni et al. (2005) observed daily movement between 2.5-36 m/d; the results of both studies were similar to the between 2-36.34 m/d observed in this study.

The previous studies also compared rate of movement between sexes and time of day. Gherardi et al. (2002) found no difference in movement between the sexes or between hours which is in contrast to the observations made during this study. The findings of this study were, however, similar to the later studies that showed that females were more nomadic than males (Barbaresi et al. 2004), and that movement was more rapid at night (Aquiloni et al. 2005).

Both of the studies by Gherardi et al. (2002) and Aquiloni et al. (2005) examined burrow preference; neither of their results are supported by the current study. In the temporary stream in Portugal, Aquiloni et al. (2005) found that bank burrows were not utilized by males and females but did find that males utilized boulders and females utilized vegetation. In the irrigation ditches of Italy, Gherardi et al. (2002) found that boulders were more often occupied but more commonly vacated than bank burrows. It was observed by Gherardi et al. (2002) that the *P. clarkii* studied spent 47-49% of their time in the open which is much higher than the 42 out of 225 relocations observed in the open in this study. This discrepancy may be due to a number of factors such as decreased predation risk for both an introduced population and the temporary nature of the bodies of water in both European studies. Another factor may have been moon phase which ranged from 93% visibility on August 10^{th} to 9% visibility on August 24^{th}. When moon visibility is the highest, it may allow for visual nocturnal predators to see the crayfish easier. This has the potential to negatively influence the crayfish rate of movements during periods of high moon visibility.

Various predators were observed at Anson Springs with two predation events occurring during this study. The first predation event appeared to be the result of an encounter with a raccoon (*Procyon lotor*) as only the chelae and radio transmitter were left on the stream bank. The second predation event was most likely a largemouth bass (*Micropterus salmoides*) because, as the signal was tracked, it would rapidly evade the tracker, a characteristic only possible from this large aquatic predator. Other potential predators in the area are wild boar (*Sus scrofa*), ringtails (*Bassariscus astutus*), Sunfish (*Lepomis sp.*), cottonmouth (*Agkistrodon piscivorus*), Common Black Hawk (*Buteogallus anthracinus*), and Great Blue Heron (*Ardea herodias*).

The abundance of predators may account for the avoidance strategies observed during this study such as moving more at night than during the day and occupying bank burrows more frequently than other burrows. Increased movement at night over the day may allow the crayfish to avoid diurnal predators such as the Common Black Hawk, Great Blue Heron, and Sunfish by taking daytime refuge in their respective refugia. It may even be extrapolated that since Form I males are moving less, they may be using their more robust body size to acquire and keep the bank burrows which Gherardi et al. (2002) hypothesized may be the more desirable burrow. This would force the less robust females to utilize whatever refugia may be available explaining their lack of preference toward any specific type of refugia. Future studies could attempt to tease out this interesting interaction to determine if populations introduced within the United States or Mexico behave more similarly to this native population or the introduced populations of Europe.

In conclusion, this study found difference between the observed movement in a native population of *P. clarkii* and the results found in previous introduced populations of *P. clarkii*. It was observed that females moved more than males. It was also observed that both males and females moved more at night than during the day. When refugia preference was considered, it was found that males were more commonly found in bank burrows while and both males and females were less often observed utilizing boulders as refugia.

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BIOGRAPHY

Michael Jacob Lucero was born in Wheeling, West Virginia on June 2nd, 1990. He resided in the nearby town of Wellsburg, West Virginia for much of his life where the Appalachian Mountains and forests served as his playground. Michael took his passion for the outdoors with him to his undergraduate career where he studied Biology at West Liberty University participating and various research projects during his time there.

After graduating, Michael worked on a cattle ranch, then as a lab technician at a midstream petroleum plant. He returned to academia to continue his biological studies at Angelo State University to attend graduate school in Biology, under the guidance of Dr. Ned Strenth. Michael continued to learn about his natural surroundings in the South-Western United States and Northern Mexico. Michael earned his Master's degree in Biology on August 2018.